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TITLE: High-Throughput Sequencing of Germline and Tumor From Men with Early-Onset Metastatic Prostate Cancer

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14. ABSTRACT

In this project, we proposed to use next generation sequencing approaches to analyze germline DNA and prostate cancer tissue from 20 men with early-onset, metastatic prostate cancer in order to identify variants that increase the risk of developing clinically significant prostate cancer, as well as novel driving somatic alterations. We have successfully, enrolled 18 men and are currently conducting whole exome sequencing on germline DNA from whole blood and comprehensive molecular analyses on formalin-fixed paraffin-embedded prostate tumors from these cases. In the coming year, we plan to complete enrollment, tissue procurement, and sequencing. We will also confirm tumor findings in a larger set of aggressive prostate cancer cases. Given the uniqueness of the patient cohort in this project, we expect that novel driving genes with germline and/or somatic variants will be identified, which can be followed up with future functional studies

15. SUBJECT TERMS
Prostate cancer, germline, somatic, susceptibility, metastatic, early-onset

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INTRODUCTION

In this project, next generation sequencing (NGS) approaches will be used to analyze the germline (from blood and/or normal prostate tissue) and prostate cancer tissue from men with newly diagnosed Stage 4 (Tx N1 or M1) prostate cancer. All patients will have *de novo* metastatic disease diagnosed with prostate cancer at or before age 60 years. Men of European and African American descent will be included in this study. The goal of this project is to identify germline variants that increase the risk of developing clinically significant prostate cancer, as well as novel driving somatic alterations. We believe that men with early-onset aggressive prostate cancer are more likely to harbor such variants.

KEYWORDS

Prostate cancer, germline, somatic, susceptibility, metastatic, early-onset

OVERALL PROJECT SUMMARY

During the second year of funding, we have made significant progress on Major Task 1: identifying and enrolling men with *de novo* metastatic prostate cancer presenting at or before age 60 years into our research project. Using a variety of recruitment methods including announcements of our study to clinicians and distribution of an IRB-approved flyer, we have consented 18 men for our study despite the delayed start due to the need for human subjects approval from both the UM and DOD USAMRMC Office of Research Protections Human Research Protection.

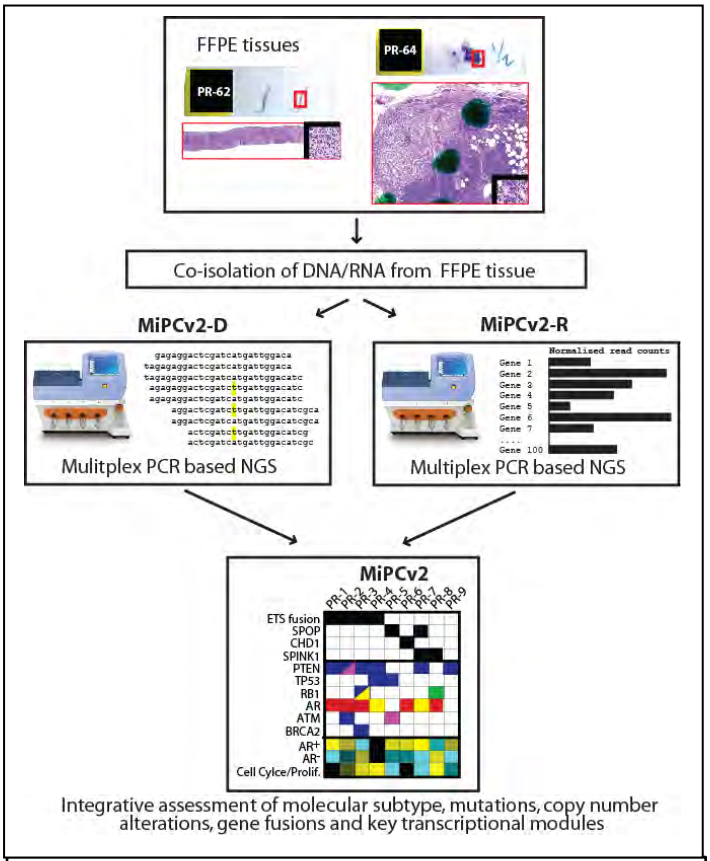


Fig 1 Schema for molecular characterization of prostate cancer samples (MiPC v.2).

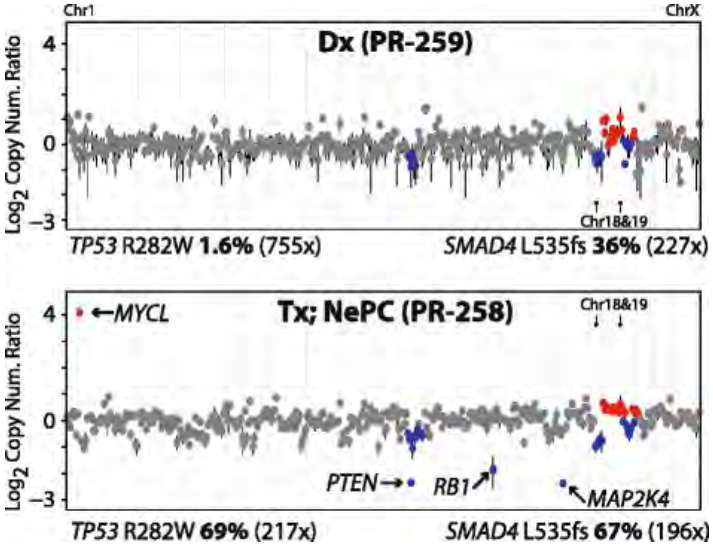
Regarding Major Tasks 2 and 3 which encompasses exome and/or transcriptome sequencing of germline DNA and prostate tumor DNA/RNA from 20 men with early-onset stage 4 prostate cancer, whole-blood has been collected and germline DNA isolated from 16 of the 18 enrolled men. Whole-exome sequencing has been conducted on 8 germline DNA samples thus far; data from these samples is currently being analyzed. In addition

to whole-blood, we have completed analysis of 5 formalin-fixed paraffin-embedded (FFPE) prostate tumor samples and we are in the process of collecting and analyzing the remaining cases.

As discussed in last year's progress report, most of the patients we are identifying for this study have already had diagnostic procedures performed which impedes our ability to collect frozen cancer specimens as initially proposed. Concurrently, Dr. Tomlins developed state of the art technologies to use FFPE prostate cancer specimens for molecular studies to characterize the prostate cancer genome and transcriptome. These include: robust protocols for co-isolating DNA/RNA from FFPE tissues, capture based NGS and qPCR approaches, and multiplexed PCR NGS based approaches. Therefore, we modified our approach to use archived FFPE biopsy and prostatectomy tumor specimen and to analyze these specimens using a qPCR/target capture DNA based NGS approaches, as well as multiplexed PCR based RNA/DNA NGS approaches to interrogate the prostate cancer transcriptome/genome. The platform, referred to as MiPC v2, based on the Oncomine Comprehensive Assay, is outlined in **Fig 1.** Likewise, in cases with intermediate amounts of DNA/RNA, we have used the Comprehensive Cancer Assay, which targets all coding exons of 400 cancer related genes (as performed for the DOD3 case below). Although we have chosen to use a targeted approach to analyze the prostate tumors, we will consider expanding our approach in some cases to include whole exome sequencing. This strategy will be used if we identify tumors that lack clear known driver mutations (e.g. ETS fusion or *PTEN* deletion).

Although much of the germline exome and tumor sequencing for this research project is still in process, we took the opportunity to perform a more comprehensive analysis of one of our more interesting patients to illustrate the molecular events that characterize the development of small cell/neuroendocrine carcinoma of the prostate (NePC). Participant DOD3 presented with metastatic prostate cancer at age 47, progressed quickly

Fig 2. Points represent the log 2 copy number ratio for all targeted genes (shown in genome order). Clonal gains and losses are shown in red and blue, respectively. Prioritized high-level copy number alterations (CNAs) alterations and somatic mutations (with variant allele frequency [%] and coverage depth [x]) are indicated. Clonal prioritized *SMAD4* mutation and SCC enriched *TP53* mutation and *MYCL*, *PTEN*, *RB1*, and *MAP2K4* copy number alterations are indicated.



with small cell carcinoma of the liver and eventually succumbed from this disease approximately one year from initial diagnosis. Using Dr. Tomlins' novel approaches to characterize small amounts of paraffin-embedded tissue, we had the opportunity to molecularly profile both the initial prostate cancer (PR-259) and the liver biopsy which contained histologically-confirmed small cell/neuroendocrine prostate carcinoma (PR-258). **Figure 2** shows the NGS genomic profiles of both cancers which demonstrates a *SMAD4* c1605delC p.L535fs frameshifting variant that was present in both PR-259 (36% variant allele frequency) and PR-258 (67% variant allele frequency). In contrast, a *TP53* c.C844T p.R282W non-synonymous variant was exclusively called in the NePC specimen (PR-258; 69% variant allele frequency). This variant was markedly enriched in PR-258, and was only present at a variant allele frequency of 1.6% (12/755 reads) in the diagnostic pre-treatment specimen (PR-259). These results are consistent with clonal origin and marked enrichment of the *TP53* R282W variant exclusively in the post-treatment NePC specimen. Exome sequencing of germline DNA isolated from white blood cells confirmed the *TP53* and *SMAD4* variants as somatic. Copy number analysis identified concordant, low-level alterations in both specimens, with focal *MYCL* amplification and homozygous *PTEN*, *RB1*, and *MAP2K4* losses identified exclusively in the NePC specimen. Integration with published genomic profiles identified *MYCL* as recurrently amplified in NePC. This is an example of an "N of 1" exceptional non-responder and highlights the ability to generate new knowledge about the molecular drivers of prostate cancer through intricate tumor and germline studies of men presenting with metastatic prostate cancer at a young age.

KEY RESEARCH ACCOMPLISHMENTS

We completed comprehensive serial molecular profiling of a patient with metastatic prostate cancer progressing to small cell carcinoma on treatment. Our findings are consistent with the concept of transdifferentiation in the development of NePC and supports the roles of *TP53* mutation, *MYCL* amplification and homozygous *PTEN*, *RB1*, and *MAP2K4* losses in the development of NePC.

CONCLUSION

We have enrolled and begun genetic analyses on a significant proportion of the planned 20 study participants with early-onset, metastatic prostate cancer. In the coming year, we plan to complete enrollment, tissue procurement, and sequencing as well as to identify genetic variants of interest. We will also study a larger set of FFPE tissues from men with high grade/aggressive prostate cancer to determine the frequency of identified variants in a larger patient cohort. Given the uniqueness of the participants in this project, we expect that novel driving genes with germline and/or somatic variants will be identified, which can be followed up with future functional studies.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Hovelson DH, McDaniel AS, Cani AK, Johnson B, Rhodes K, Williams PD, Bandla S, Bien G, Choppa P, Hyland F, Gottimukkala R, Liu G, Manivannan M, Schageman J, Ballesteros-Villagrana E, Grasso CS, Quist MJ, Yadati V, Amin A, Betz B, Knudsen KE, Cooney KA, Feng FY, Roh MH, Nelson PS, Liu C, Beer DG, Wyngaard P, Sadis S, Rhodes DR, Tomlins SA. Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. Neoplasia 17(4):385-99, 2015.

Kadakia KC, Tomlins SA, Sanghyi SK, Cani AK, Omata K, Hovelson DH, Liu CJ, Cooney KA. Comprehensive serial molecular profiling of an "N of 1" exceptional non-responder with metastatic prostate cancer progressing to small cell carcinoma on treatment. J Hematol Oncol 8:109, 2015.

INVENTIONS, PATENTS AND LICENSES Nothing to report

REPORTABLE OUTCOMES Nothing to report

OTHER ACHIEVEMENTS Nothing to report

REFERENCES None

APPENDIX A. Recruitment brochure and flyer.

About the Study

This study, officially titled "High Throughput Sequencing of Germline and Tumor from Men with Early-Onset Metastatic Prostate Cancer" will analyze blood, normal prostate tissue, and prostate cancer tissue from men with newly diagnosed Stage 4 prostate cancer at age 60 or younger.

We hope to identify genetic events that contribute to aggressive disease.

Men of all racial and ethnic backgrounds will be included in the study.

Eligibility Questions?

Study Coordinator
Prostate Cancer Genetics Project– DOD
7436 CC, 1500 E. Medical Center Drive
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734-936-2031 (phone)
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MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

High Throughput Sequencing of Germline and Tumor from Men with

Early-Onset Metastatic

Prostate Cancer

University of Michigan

Comprehensive Cancer Center

WHO CAN HELP?

Men with newly diagnosed advanced prostate cancer (T4 and/or N1 and/or M1) at age 60 or younger who are

- Undergoing standard of care surgeries or procedures

OR

- Have prostate cancer tissue available for molecular evaluation

WHAT IS INVOLVED?

- Signing a consent form
- Completing a basic questionnaire
- Providing a small blood sample

Traveling to Ann Arbor is not required.

Participation is completely voluntary and confidential and involves no cost, and the decision to participate in no way affects treatment.

Why is this important?

Prostate cancer is the *second leading cause of cancer deaths* in men the U.S.

Early-onset prostate cancer in a family member has been shown to increase risk of prostate cancer in other family members.

Individuals diagnosed with early-onset cancers are much more likely to have a genetic component to their disease.

Volunteer Today!

For eligibility questions, contact
Study Coordinator
734-936-2031 (phone)
734-647-4338 (fax)
Pcgp-project@med.umich.edu

For clinical questions, contact
Kathleen A. Cooney, MD
kcooney@med.umich.edu

Scott Tomlins, MD, PhD
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MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

HIGH THROUGHPUT SEQUENCING OF GERMLINE AND TUMOR FROM MEN WITH EARLY-ONSET, METASTATIC PROSTATE CANCER

This study will analyze blood, normal prostate tissue, and prostate cancer tissue from men with newly diagnosed Stage 4 prostate cancer to identify genetic events that contribute to aggressive disease.

Who can help?

Men with newly diagnosed advanced prostate cancer (T4 and/or N1 and/or M1) at age 60 or younger who are:

- Undergoing standard of care surgeries or procedures
  OR
- Have prostate cancer tissue available for molecular evaluation

What is involved?

- Signing a consent form
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University of Michigan
Medical School

		(n=20)	
Subtask 2 B. Complete exome sequencing, alignment and variant calling	3-30	Scott Tomlins, MD/PhD	
Subtask 2 C. Prioritize variants for followup	13-30	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 2 D.Genotype top candidate variants in prostate cancer cases (~5000 from UM and Johns Hopkins) and controls (~1500 men without prostate cancer from Johns Hopkins University)	18-36	Kathleen Cooney, MD	
Milestone(s) Achieved:			
Major Task 3: To conduct exome/transcriptome analyses on prostate cancer tumor from 20 study participants with metastatic prostate cancer diagnosed at or before age 60.	**Months** 3-36	**University of Michigan**	**North Carolina**
Subtask 3 A. Collect sample(s) of prostate cancer from 20 study participants. For those men who are having a surgical procedure, extra tumor not required for clinical purposes will be used. For the remaining participants, formalin-fixed paraffin-embedded (FPPE) tumor will be obtained from prior diagnostic or therapeutic procedures	3-27	Kathleen Cooney, MD and Scott Tomlins MD/PhD	
Subtask 3 Complete tumor exome and transcriptome sequencing, including analysis of copy number, gene-fusions, and outliers	3-30	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 3 Prioritize potential candidate mutations for follow up using driver analysis pipeline	13-36	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 3 Test proposed driver mutations in a set of 100 FPPE prostate cancer specimens	18-36	Scott Tomlins, MD/PhD	

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the following table.

	Year 1				**Year 2**				**Year 3**
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**
Site 1	3	2	3	2	3	2	3	2	
Target Enrollment (cumulative)	**3**	**5**	**8**	**10**	**13**	**15**	**18**	**20**	
									No Subject Recruitment – Follow up only

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